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Analyzation of Metabolic Reprogramming in Drug-Resistant MCF-7 Cells

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Introduction

There are two types of metabolic pathways utilized to obtain energy: anaerobic glycolysis and oxidative phosphorylation. In anaerobic glycolysis, the start molecule is glucose, and the end product is lactic acid. Oxidative phosphorylation also starts with glucose, but converts it into pyruvate, which then enters the tricarboxylic acid cycle, followed by the electron transport chain. Cancer cells prefer to use anaerobic glycolysis as their means of obtaining energy, even in the presence of oxygen. This phenomenon is known as the Warburg effect, where cancer cells go through aerobic glycolysis—following the anaerobic glycolysis pathway even in the presence of oxygen. By utilizing anaerobic glycolysis, cancer cells are able to obtain energy much faster as opposed to when using aerobic glycolysis, which explains a tumor's capability of proliferating at a rapid pace.[2]

Chemotherapy is one method of treating cancer, but does not guarantee curing it, for the treatment sometimes does not kill all the cells, which may cause the cancer to regress. Understanding the change in metabolism of these drug-resistant cancer cells provides a potential target for cancer treatment, with the possibility of curing cancer. The goal of this study is to simulate dormancy in the MCF-7 cell line, a form of breast cancer, and discover the key differences in its metabolism in comparison to the native wildtype cells. In this study, doxorubicin was the chemotherapy drug utilized to create drug-resistant MCF-7 cell lines, which prevents cell growth by proteolytically cleaving cAMP responsive element binding protein 3-like 1 (CREB3L1), a transcription factor, via the production of ceramide.[3]

Cell Culturing

Cells were grown in a T-75 flask with RPMI media containing 10% FBS and 1% penicillin/strep, and stored in an incubator under 37°C. Cells that were to be treated with drug were trypsinized and transferred to 25-cm small plates. Doxorubicin was dosed in concentrations of 1 μM, 2 μM, 2.5 μM, 3 μM, 4 μM, and 5 μM. Cell cultures were refreshed with new RPMI media whenever the media (originally orange) turned yellow, an indicator that the cell culture had consumed all of the nutrients in the media and that the culture was slowly becoming acidic. When the confluency of the small plates was full, meaning, when the surface of the plate was covered with cells, and there were no empty spaces, 1 mL of trypsin was used to loosen cells from the plate surface. To neutralize the trypsin, 4 mL of RPMI media was used. 1 mL of the total solution was then passed to a new small plate, with an additional 4 mL of RPMI media to continue the culture at 20% plate confluency. In T-75 flasks, dosed cultures that were stable in confluency for three days were scraped, collected, and frozen for future use for the MTT assay.

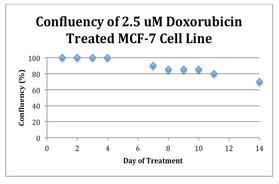

***Figure 1**: A time-progressive graph of the confluency of MCF-7 cells treated with 2.5 μM Doxorubicin*

Treated cells were considered to be drug-resistant if its cell confluency initially declined, but then stabilized at a lower percentage. We refer to this stage as "dormancy". We discovered that doxorubicin was most effective in achieving dormancy with a concentration range of 2uM – 3 μM, although cells tended to die off approximately a week after initial treatment. **Figure 1** showed the progress in confluency of one of the cell treatments, with 2.5 μM doxorubicin. During the weekends, confluency was not recorded. This cell line was used in the MTT assay conducted for this study. Cells were hard to maintain because it was difficult to predict if cells would proliferate or die. The data obtained from the treated cells could not explain the MCF-7 cell line's behavior in the presence of doxorubicin. However, it had been noticed that if drug treatment was started at a high confluency, it would take much longer before the cells began to show a decline in cell population.

Doxorubicin Treatments

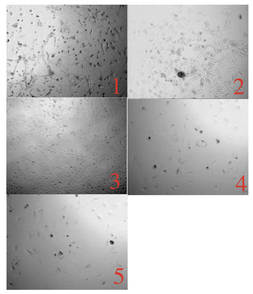

***Figure 2**: Morphology observations of doxorubicin-treated MCF-7 cells. 1: Wildtype MCF-7 cell line. 2: 1 μM Doxorubicin-treated MCF-7 cell line. 3: 2.5 μM Doxorubicin-treated MCF-7 cell line. 4: 5 μM Doxorubicin-treated MCF-7 cell line. 5. 10 μM Doxorubicin-treated MCF-7 cell line.*

Based on **Figure 2**, the treatments showed that doses higher than 5uM were too strong to create drug-resistant cancer cell lines, as all of the cells died at a concentration of 10 μM. However, doxorubicin doses of 1uM and 2.5uM caused the cells to exhibit a morphology change. As opposed to the more rounded shape of MCF-7 cells in wildtype form, cells treated with concentrations of either 1uM or 2.5uM became more elongated and thinner at the ends, while the middle of the cells remained round. We believe that this change in morphology is what allows MCF-7 cells to adapt in the presence of doxorubicin, so that they have a higher chance of staying alive.

MTT Assay

MTT Assay was conducted to test for the toxicity and therefore viability of the cell lines as followed by a protocol from Waller and Provost Lab. This assay involves reducing 30(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (yellow MTT) to a purple formazon in the mitochondria of alive cells. Mitochondrial dehydrogenases cleave the tetrazolium ring in the mitochondria in order create purple MTT formazan crystals, which are insoluble without the addition of another solvent. DMSO was used to solubilize the formazan crystals.

	WT				2.5 uM				5 uM		
Control	WT Control (1)	WT Control (2)	Wt Control (3)		2.5 Control (1)	2.5 Control (2)	2.5 Control (3)		5 Control (1)	5 Control (2)	5 Control (3)
2.5 uM	WT Dosed 2.5 (1)	WT Dosed 2.5 (2)	WT Dosed 2.5 (3)		2.5 Dosed 2.5 (1)	2.5 Dosed 2.5 (2)	2.5 Dosed 2.5 (3)		5 Dosed 2.5 (1)	5 Dosed 2.5 (2)	5 Dosed 2.5 (3)
5 uM	WT Dosed 5 (1)	WT Dosed 5 (2)	WT Dosed 5 (3)		2.5 Dosed 5 (1)	2.5 Dosed 5 (2)	2.5 Dosed 5 (3)		5 Dosed 5 (1)	5 Dosed 5 (2)	5 Dosed 5 (3)
10 uM	WT Dosed 10 (1)	WT Dosed 10 (2)	WT Dosed 10 (3)		2.5 Dosed 10 (1)	2.5 Dosed 10 (2)	2.5 Dosed 10 (3)		5 Dosed 10 (1)	5 Dosed 10 (2)	5 Dosed 10 (3)
DMSO Control	DMSO Control 1	DMSO Control 2									

***Table 1**: Setup of the 96-well plate for the MTT assay.*

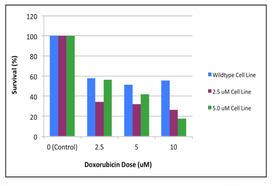

***Figure 3**: Results from the MTT assay.*

The setup for the MTT assay is shown in **Table 1**. Using a 96-well plate, 36 wells were used in the assay to test 2.5 μM and 5.0 μM doxorubicin-treated cells in order to determine whether or not the treated cells were drug resistant. Along with a control, each type of cell line was tested in triplicates. The three cell lines were given four different types of treatments: control, 2.5 μM, 5 μM, and 10 μM doxorubicin. Each well was aimed to have 2,500 cells/mL, and contained a volume of 200 uL. After setting up the well plate, cells were given one day to acclimate to the new environment before treating with various concentrations of doxorubicin. Once cells were incubated for another day, 20 mL of 5 g/mL thiazolyl blue tetrazolium bromide (MTT solution) was added to each well. After ten minutes of gentle shaking, each well's absorbance was recorded via spectrophotometer at 600 nm.[8]

It was predicted that the treated cell lines would exhibit resistance to cell dosage. Meaning, the 2.5 μM cell line should have shown enhanced survival in comparison to the other cells when treated with 2.5 μM doxorubicin. However, the data has shown that in all dosage treatments, the wildtype cells survived more than the treated cell lines. Because this assay was also performed only once, a conclusion cannot be made with this data. However, this experiment suggests that the treated cell lines, though not drug-resistant, were more weakened to the cell treatment, meaning the treated cell lines were more affected by the drug in comparison to wildtype.

Discussion

This study was conducted for ten weeks; for three of those ten weeks, there was a contamination problem in the cell incubator, thus compromising most cells with yeast growth. Due to this contamination issue and the limited amount of time to conduct research, it was difficult to maintain growth of MCF-7 cells, and observe the confluency trend of treated cells. This issue was resolved towards the last two weeks of research. The figures shown in this report reflect data from MCF-7 cells that were uncontaminated. Due to the contamination, there was a lack of several viable cell lines available for analysis and documentation in this report

We plan to continue treating MCF-7 cells with doxorubicin in order to create drug-resistant cells and find a more concrete dosage range of which doxorubicin can be used to create said cell lines, as well as confirm the findings from the MTT assay, which suggest the cell lines made were not resistant, only weakened. These cell lines will then be measured via oxygen electrode in order to further analyze the mitochondria activity of these cells by measuring the amount of aerobic respiration, and to compare them to wildtype MCF-7 cells. Future MTT assays will be done with cell lines that have had stable confluency for a longer period of time longer than three days. When it is confirmed through MTT assays and the oxygen electrode that drug resistant cell lines were successfully created, cells will then be analyzed by electron microscope in order to obtain a more accurate visual in terms of morphological changes in MCF-7 cells. From there, we intend to discover the same findings with another drug, cyclophosphamide, in order to see if different chemotherapy agents would require different concentrations to make MCF-7 cells drug resistant, as well as if similar or different morphological changes can be observed.

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